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THE USE OF LARGER QUANTITIES OF BLOOD SERUM IN THE WASSERMANN REACTION

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The use of large quantities of blood serum in performing the Wassermann reaction has been advocated by others. For some reason it has not received the attention nor support that it deserves. Bohan and Lynch¹ showed conclusively the superiority of large quantities over small quantities of blood serum.

The following report deals with 50 serums, 15 of which came from nonsyphilitic persons, and 35 from syphilitics under treatment.

Method.—The Wassermann method with human hemolytic system was used in all the tests. Human serum, complement, antigen, hemolytic amboceptor and blood corpuscles were diluted with physiologic salt solution so that 0.25 c.c. of diluted ingredient represented the test dose, which brought the total quantity in each test tube up to 1.25 c.c.

Antigen.—The antigen was alcoholic extract of human heart muscle. The largest quantity that was not anticomplementary was used as the test dose.

Hemolytic Amboceptor.—The blood serum of a rabbit that had been immunized against washed human blood corpuscles was used in doses of from 1 to 2 units. The term unit was applied to the smallest quantity of hemolytic serum which with 0.025 c.c. of complement dissolved the test dose of blood corpuscles in 1 hour.

Corpuscles.—Human corpuscles were well washed and were used in doses of 0.25 c.c. of a 2.5% suspension in physiologic salt solution.

Complement.—The blood serum of guinea-pigs was used in doses of 0.05, 0.025 and 0.0125 c.c.

The human serums were heated to 56 C. for 30 minutes. Six test tubes, 1, 2, and 3 as antigen tubes, and 1', 2', and 3' as control tubes, were used in each test. Each of the 6 tubes received 0.25 c.c. of diluted human serum. Each of the 1st pair of tubes, Tubes 1 and 1', received 0.25 c.c. of 1:5 dilution of complement serum; each of the 2nd pair of tubes, Tubes 2 and 2', received 0.25 c.c. of 1:10 dilution of complement serum; and each of the 3rd pair of tubes received 0.25 c.c. of 1:20 dilution of complement serum.

Each of the antigen tubes, Tubes 1, 2, and 3, received 0.25 c.c. of diluted antigen; and the control tubes, Tubes 1', 2', and 3', received 0.25 c.c. of physiologic salt solution each. These mixtures were placed in the incubator at 37 C. for 1 hour. After having been in the incubator 1 hour each tube received 0.5 c.c. of sensitized corpuscles representing 0.25 c.c. of 2.5% suspension of corpuscles and 0.25 c.c. of diluted hemolytic amboceptor which were mixed

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¹ Jour. Am. Med. Assn., 1917, 69, p. 1220.

30 minutes before they were added to the contents of the tubes. After shaking, the tubes were returned to the incubator for another hour during which time they were shaken 3 or 4 times. After an hour in the incubator the tubes were allowed to stand at room temperature for 3 or 4 hours, after which the results were read and recorded.

TEST 1

Serums 1 to 10, inclusive, were obtained from nonsyphilitic persons. Within 24 hours after the bleeding these serums were tested in quantities of 0.05 c.c. and 0.25 c.c. per test tube.

TABLE 1

WASSERMANN REACTION WITH VARYING QUANTITIES OF FRESH NONGLYCEROLATED HUMAN SERUM

No. of Serum	Quantity of Serum per Tube C.c.	Quantity of Amboceptor per Tube Unit	Readings*						Results
			Antigen Tubes			Control Tubes			
			1	2	3	1'	2'	3'	
1	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
2	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
3	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
4	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
5	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
6	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
7	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
8	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
9	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative
10	0.05	1.0	+	+	±	+	+	±	Negative
	0.25	1.0	+	+	tr	+	+	tr	Negative

* Explanation: tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

Table 1 shows the results obtained with the first 10 serums. The larger quantities of serum gave results identical with those obtained with the smaller quantities and were but slightly anticomplementary. These larger quantities did not give false positive results.

TEST 2

Serums 11 to 15 inclusive, were obtained from nonsyphilitic persons and serums 16 to 20, inclusive, came from syphilitics under treatment.

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Each serum was divided into 2 portions, A and B. Portion A remained untreated and Portion B was mixed with an equal volume of sterilized Merck's blue label glycerol. Each portion was tested in quantities of 0.05 c.c. and 0.125 c.c. of serum (0.1 c.c. and 0.25 c.c. of serum-glycerol mixture) per test tube.

TABLE 2

WASSERMANN REACTION WITH VARYING QUANTITIES OF FRESH NONGLYCEROLATED AND GLYCEROLATED HUMAN SERUM

No. of Serum	Portions: A = Nongly-cerated, B = Gly-cerated	Quantity of Serum per Tube, C.c.	Quantity of Ambo-ceptor per Tube Unit	Readings*						Results
				Antigen Tubes			Control Tubes			
				1	2	3	1'	2'	3'	
11	A	0.05	1.0	+	+	±	+	+	±	Negative
	A	0.125	1.0	+	+	±	+	+	±	Negative
	B	0.05	1.25	+	+	±	+	+	±	Negative
	B	0.125	1.5	+	+	±	+	+	±	Negative
12	A	0.05	1.0	+	+	±	+	+	±	Negative
	A	0.125	1.0	+	+	±	+	+	±	Negative
	B	0.05	1.25	+	+	±	+	+	±	Negative
	B	0.125	1.5	+	+	±	+	+	±	Negative
13	A	0.05	1.0	+	+	±	+	+	±	Negative
	A	0.125	1.0	+	+	±	+	+	±	Negative
	B	0.05	1.25	+	+	±	+	+	±	Negative
	B	0.125	1.5	+	+	±	+	+	±	Negative
14	A	0.05	1.0	+	+	±	+	+	±	Negative
	A	0.125	1.0	+	+	±	+	+	±	Negative
	B	0.05	1.25	+	+	±	+	+	±	Negative
	B	0.125	1.5	+	+	±	+	+	±	Negative
15	A	0.05	1.0	+	+	±	+	+	±	Negative
	A	0.125	1.0	+	+	±	+	+	±	Negative
	B	0.05	1.25	+	+	±	+	+	±	Negative
	B	0.125	1.5	+	+	±	+	+	±	Negative
16	A	0.05	1.0	+	+	±?	+	+	±	Faintly positive, ±
	A	0.125	1.0	+	+	tr	+	+	±	Weakly positive, +
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +
	B	0.125	1.5	+	±	0	+	+	±	Strongly positive, +++
17	A	0.05	1.0	+	+	±?	+	+	±	Faintly positive, ±
	A	0.125	1.0	+	+	tr	+	+	±	Weakly positive, +
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +
	B	0.125	1.5	+	±	0	+	+	±	Strongly positive, +++
18	A	0.05	1.0	+	+	tr	+	+	±	Weakly positive, +
	A	0.125	1.0	+	+	0	+	+	±	Moderately positive, ++
	B	0.05	1.25	+	+	0	+	+	±	Moderately positive, ++
	B	0.125	1.5	+	tr	0	+	+	±	Strongly positive, ++++
19	A	0.05	1.0	+	+	tr	+	+	±	Weakly positive, +
	A	0.125	1.0	+	+	0	+	+	±	Moderately positive, ++
	B	0.05	1.25	+	+	tr	+	+	±	Weakly positive, +
	B	0.125	1.5	+	tr	0	+	+	±	Strongly positive, ++++
20	A	0.05	1.0	+	+	0	+	+	±	Moderately positive, ++
	A	0.125	1.0	+	±	0	+	+	±	Strongly positive, +++
	B	0.05	1.25	±	+	0	+	+	±	Moderately positive, ++
	B	0.125	1.5	±	0	0	+	+	±	Strongly positive, ++++++

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

Table 2 shows that serums 11 to 15, inclusive, gave negative results with all of the four methods. Serums 16 to 20, inclusive, gave positive results. The larger quantities of serum gave stronger positive results than did the smaller quantities, and the glycerolated portions gave stronger positive results than did the nonglycerolated portions. This was particularly true of the larger quantities of the glycerolated portions, which reacted much stronger than did corresponding quantities of the nonglycerolated portions. Glycerol being anticomplementary, the larger quantities required larger quantities of hemolytic amboceptor.

TEST 3

Thirty old glycerolated serums were tested in quantities of 0.05 and 0.125 c.c. per test tube.

TABLE 3

WASSERMANN REACTION WITH VARYING QUANTITIES OLD GLYCEROLATED HUMAN SERUM

No. of Serum	Quantity of Serum per Tube, C.c.	Quantity of Amboceptor per Tube, Unit	Readings*						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
21	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	+	Negative	
22	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
23	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
24	0.05	1.5	+	+	±?	+	+	±	Faintly positive,	±
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
25	0.05	1.5	+	+	±?	+	+	±	Faintly positive,	±
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
26	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	0	+	+	tr	Weakly positive,	+
27	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	tr	Negative	
28	0.05	1.5	+	+	tr	+	+	±	Weakly positive,	+
	0.125	2.0	+	±	0	+	+	tr	Moderately positive,	++
29	0.05	1.5	+	+	0	+	+	±	Moderately positive,	++
	0.125	2.0	+	0	0	+	+	tr	Strongly positive,	++++
30	0.05	1.5	+	+	0	+	+	±	Moderately positive,	++
	0.125	2.0	+	0	0	+	+	tr	Strongly positive,	++++
31	0.05	1.5	+	+	±	+	+	±	Negative	
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+
32	0.05	1.5	+	±	tr	+	+	tr	Faintly positive,	±
	0.125	2.0	+	+	tr	+	+	±	Weakly positive,	+

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

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TABLE 3—Continued

WASSERMANN REACTION WITH VARYING QUANTITIES OLD GLYCEROLATED HUMAN SERUM

No. of Serum	Quantity of Serum per Tube, C.c.	Quantity of Amboceptor per Tube, Unit	Readings*						Results	
			Antigen Tubes			Control Tubes				
			1	2	3	1'	2'	3'		
33	0.05 0.125	1.5 2.0	+	+	tr	+	+	tr	Negative Weakly positive,	+
34	0.05 0.125	1.5 2.0	+	+	tr	+	+	+	Negative Weakly positive,	+
35	0.05 0.125	1.5 2.0	+	+	tr	+	+	tr	Negative Weakly positive,	+
36	0.05 0.125	1.5 2.0	+	+	tr	+	+	tr	Negative Strongly positive,	+++
37	0.05 0.125	1.5 2.0	+	+	tr?	+	+	tr	Faintly positive, Strongly positive,	± +++++
38	0.05 0.125	1.5 2.0	+	+	0	+	+	tr	Weakly positive, Strongly positive,	+ +++++
39	0.05 0.125	1.5 2.0	+	+	0	+	+	tr	Weakly positive, Strongly positive,	+ ++++++
40	0.05 0.125	1.5 2.0	+	±	0	+	+	tr	Moderately positive, Strongly positive,	++ ++++++
41	0.05 0.125	1.5 2.0	+	+	0	+	+	tr	Weakly positive, Strongly positive,	+ +++
42	0.05 0.125	1.5 2.0	+	tr	0	+	+	tr	Strongly positive, Strongly positive,	+++ +++++
43	0.05 0.125	1.5 2.0	+	0	0	+	+	tr	Strongly positive, Strongly positive,	++++ ++++++
44	0.05 0.125	1.5 2.0	+	tr	0	+	+	tr	Strongly positive, Strongly positive,	+++ ++++++
45	0.05 0.125	1.5 2.0	+	tr	0	+	+	tr	Strongly positive, Strongly positive,	+++ +++++
46	0.05 0.125	1.5 2.0	+	±	0	+	+	tr	Moderately positive, Strongly positive,	++ +++++
47	0.05 0.125	1.5 2.0	+	±	0	+	+	tr	Moderately positive, Strongly positive,	++ ++++++
48	0.05 0.125	1.5 2.0	+	tr	0	+	+	tr	Strongly positive, Strongly positive,	+++ ++++++
49	0.05 0.125	1.5 2.0	+	tr	0	+	+	tr	Strongly positive, Strongly positive,	+++ ++++++
50	0.05 0.125	1.5 2.0	+	0	0	+	+	tr	Strongly positive, Strongly positive,	++++ ++++++

* Explanation: 0 = no hemolysis; tr = hemolysis less than 50 %; ± = hemolysis between 50 and 100 %; + = complete hemolysis.

The results are shown in Table 3. The larger quantities of serum gave much stronger positive results than did the smaller quantities. With Serums 36, 37, and 39 the differences were unusually marked. Of Serum 36 the smaller quantity gave a negative result, and the larger

quantity gave a strongly positive result. Serum 37 gave faintly positive ($\pm$) and strongly positive (+++++) results, while Serum 39 gave + and ++++++. These results are slightly out of proportion because the larger quantities of serum were only $2\frac{1}{2}$ times as large as the smaller quantities.

CONCLUSIONS

Of fresh serum quantities amounting to 0.2 of the total volume per test tube did not give false positive results and were but slightly anti-complementary.

With larger quantities of serum a higher percentage of positive results is obtained.

Glycerol is anticomplementary; the anticomplementary action may be overcome by increasing the quantity of amboceptor.

Unconditional negative results should not be reported unless fairly large quantities of serum were used in the test.